## REMARKS

Claims 123-124, 126-129, 131 and 133-159 are pending in the application. Applicant acknowledges renumbering of claims 126-129 as 125-128, claim 131 as claim 129, and claims 133-159 as claims 130-156. Claims 139-156 have been withdrawn from consideration. Claims 123-138 have been rejected.

Claim 123 has been objected to. Claims 123-138 have been rejected under 35 USC 112. Claims 129-137 have been rejected under 35 USC 102(a) as anticipated by Dvir et al. Claim138 has been rejected under 35 USC 103(a) as being obvious over Dvir et al. Claims 125-128 and 130-132 have now been canceled. Claims 123, 124, 129 and 133 have now been amended.

## Status of the Application

Applicant thanks the Examiner for renumbering of claims as detailed in the Office Action, and acknowledges the revised claim numbers.

## Priority

The Examiner has asserted that while PCT IL/04/00335 clearly provides support for the subject-matter of claims 123-128, 129-137 and 141, provisional application 60/463, 049 fails to provide support for the subject-matter of claims 123, 125, 127, 128, 129, 130-132 and 133-138. Further, the Examiner has asserted that while foreign priority document IL 156273 provides support for the subject-matter of claims 123-128, IL 156273 fails to provide support for the subject-matter of claims 129-138. Applicant disagrees.

IL 156273 discloses the purification of partially deglycosylated glucocerebrosidase, crystallization of the purified enzyme and generation of accurate structure coordinates defining a 3-dimensional structure of the partially deglycosylated enzyme preparation. Pharmaceutical compounds having the glucocerebrosidase as an active ingredient, for the treatment and prevention of glucocerebrosidase deficiency, are also disclosed.

Claims 123, 124, 129 and 133 have now been amended. Claims 125-128, 130-132 have been canceled, without prejudice. Amended claim 129 is drawn to a purified preparation of glucocerebrosidase molecules having the following characteristics:

(i) an amino acid sequence as set forth in SEQ ID NO: 1;

(ii) glycosylation at Asn 19;

(iii) unglycosylation at one or more of glycosylation residues Asn 59, Asn 146 and Asn 270 of SEQ ID NO: 1;

(iv) ability to form pure crystals having X-ray diffraction capacity enabling generation of structural coordinates as set forth in Table 4 and having unit cell dimensions of a = about 107.7 angstroms, b = about 285.2 angstroms and c = about 91.8 angstroms and a crystal space group of $C222_1$.

IL 156273 teaches partial deglycosylation, purification and crystalization of human glucocerebrosidase:

> "According to still further features in the described preferred embodiments, an amino acid sequence of the glucocerebrosidase molecule comprises a first N-linked glycosylation consensus sequence, wherein the first N-linked glycosylation consensus sequence is attached to a sugar moiety comprising a monosaccharide or a disaccharide directly attached to the first N-linked glycosylation consensus sequence, and whereas the step of partially deglycosylating the glucocerebrosidase molecule is effected so as to leave the monosaccharide or the disaccharide attached to the first N-linked glycosylation consensus sequence." (page 5, lines 4-11)

> "Preferably, the glucocerebrosidase molecule of the present invention has a partially glycosylated amino acid sequence.
> As used herein, the phrase "partially glycosylated amino acid sequence" refers to an amino acid sequence of a glucocerebrosidase molecule of the present invention having a glycosylation pattern in which at least one N-linked glycosylation consensus sequence thereof which is naturally glycosylated is non-glycosylated. It will be understood by the ordinarily skilled artisan that N-linked glycosylation consensus sequences of the amino acid sequence of glucocerebrosidase which are normally glycosylated are located at positions 19, 59, 146, and 270 of the amino acid sequence (for example, refer to Berg-Fussman A. *et al.*, 1993. J Biol Chem. 268:14861-14866).
> Preferably, the partially glycosylated amino acid sequence comprises a sugar moiety attached to the first N-linked glycosylation consensus sequence thereof.
> As used herein, the term "first" when referring to an N-linked glycosylation consensus sequence of an amino acid sequence corresponds to the N-linked glycosylation consensus sequence of the amino acid sequence located closest to the amino-terminal of the amino acid sequence.

For example, in the amino acid sequence set forth in SEQ ID NO: 1 or 8, the first N-linked glycosylation consensus sequence is located at position 19 of the amino acid sequence."(page 41, lines 5-22)

*"Crystallization:* A 5 mg aliquot of Cerezyme®, a recombinant variant of human glucocerebrosidase having the natural Arg495 residue substituted with a His residue, and oligosaccharide chains of glycosylated N-linked glycosylation sites modified to terminate in mannose sugars, was dialyzed overnight against phosphate-buffered saline (PBS) pH 7.0 and deglycosylated by incubation at 25 °C for 88 hours with 150 units N-glycosidase F." (page 55, lines 11-16)

*"Experimental Results:*
Following extensive empirical experimentation, highly purified, highly ordered human glucocerebrosidase polypeptide crystals were successfully grown. X-ray crystallographic analysis of such crystals generated Protein DataBank (PDB) a set of structure coordinates defining the essentially complete 3D atomic structure of human glucocerebrosidase polypeptide at 2.0 angstroms (Table 4, enclosed CD-ROM)".(page 57, lines 10-16)

"A set of structure coordinates defining the predicted structure of the set of amino acid residues predicted to have at least one atom positioned within 10 angstroms of at least one atom of a mutated amino acid were also identified for each of the aforementioned human glucocerebrosidase mutants associated with Gaucher disease. The structure of such a set of amino acid residues located within 10 angstroms of the mutated amino acid residue is hereinafter referred to as "10-angstrom radius structure". A similar 10-angstrom radius structure was defined for each mutation within the structure of the non-mutated enzyme. These non-mutated 10-angstrom radius structures are defined by sets of Table 4 (enclosed CD-ROM) coordinates defining the structures of sets of amino acid residues from the amino acid sequence of the non-mutated glucocerebrosidase molecule located at the same positions within the amino acid sequence of the non-mutated glucocerebrosidase molecule (SEQ ID NO: 1) as the amino acid residues included in the 10-angstrom radius structures of the corresponding mutant glucocerebrosidase molecule within the amino acid sequence of the mutated glucocerebrosidase molecule."(page 63, lines 6-18)

Claim 133 reads on the glucocerebrosidase preparation of claim 129, having catalytic activity similar to that of fully glycosylated glucocerebrosidase. Such

glucocerebrosidase is disclosed in the instant specification, for example, on page 15, lines 13-17:

> "While reducing the present invention to practice, the present inventors succeeded in crystallizing a human glucocerebrosidase molecule having normal enzymatic activity of which crystallographic analysis was used for successfully generating for the first time a set of structure coordinates defining the essentially complete structure of the crystallized glucocerebrosidase molecule at atomic resolution. The present inventors further used this set of structure coordinates to generate for the first time sets of structure coordinates defining with optimally high resolution and accuracy essentially complete predicted 3D structures of mutant glucocerebrosidase molecules associated with Gaucher disease, including mutant portions thereof. While conceiving the present invention, the present inventors hypothesized that such sets of structure coordinates defining predicted 3D structures of mutant glucocerebrosidase molecules, in particular mutant portions thereof, could be used for identifying a compound capable of correcting *in-vivo*, with optimal efficacy and safety, the impaired enzymatic activity of a mutant glucocerebrosidase molecule associated with Gaucher disease."(page 15, lines 13-17)

Further support for the limitations of claim 133 can be found throughout the Examples, specifically as illustrated in Figs. 1b, 1c and 1d; and Figs. 2a and 2b.

Claim 134 reads on a glucocerebrosidase of claim 129, with the further limitation of an exposed mannose residue, as disclosed in Example 1 of the instant specification (see "Crystallization", *supra*).

Claims 136-138 read on pharmaceutical compositions and articles of manufacture comprising the glucocerebrosidase preparation of claim 129. The subject-matter of these claims is disclosed in IL 156273 (see page 26, lines 23-28):

> The compound of the present invention can be used *per se* or it can be formulated as the active ingredient of a pharmaceutical composition comprising suitable carriers and/or diluents, and an effective concentration of the compound of the present invention so as to be suitable for therapeutically correcting the impaired enzymatic activity of the mutant glucocerebrosidase molecule when administered to a subject in need thereof, in particular a subject having Gaucher disease."(page 26, lines 23-28)

Thus, Applicants submit that the limitations of amended claim 129, and claims dependent therefrom, are fully supported in IL 156273, and should therefore be accorded priority therefrom.

***Oath/Declaration***

An executed oath/declaration complying with the requirements of 37 CFR 1.67(a), will follow shortly under separate cover.

***Specification/Informalities***

The title of the instant specification has been amended to "Partially Deglycosylated Glucocerebrosidase Polypeptide and Crystals Thereof", as suggested by the Examiner.

Regarding the sequence listing, please insert into the Specification the substitute listing paper copy of the sequence listing provided on January 4, 2007 after the Abstract.

Regarding embedded hyperlinks, the Specification has now been amended to delete all embedded hyperlinks and/or other forms of browser-executable code, as detailed *supra*.

Regarding the identity of the duplicate CD-ROMs filed with the specification on October 4, 2005, Applicant hereby declares that the content of the enclosed duplicate CD-ROMs is identical and includes no new matter.

Regarding the reference statement in the specification, according to the suggestion generously provided by the Examiner, the specification has now been amended to include the statement "The material included in the CD ROM is hereby entirely incorporated into the specification by reference."(see Amendments to the Specification", *supra*).

***Claim Objections***

Regarding claim 123, the term "glycolated" has been replaced by the correctly spelled term "glycosylated".

***35 U.S.C. § 112, Second Paragraph Rejections***

The Examiner has rejected claims 124, 127 and 129-138 as being indefinite for failing to particularly point out and distinctly claim the subject matter which the

applicant regards as the Invention. Claims 127 and 130-132 have now been canceled, rendering moot the Examiner's rejection thereof. Claims 124, 129 and 133 have now been amended.

Regarding claim 124, the phrase "resolution of 2.9 angstroms or higher", which clearly relates to a resolution of 2.9 angstroms or a greater resolution, has now been replaced with the phrase "resolution of 2.9 angstroms or greater resolution", further defining and clarifying the claimed composition of matter.

Claim 129 has now been amended to incorporate the limitations of now canceled claims 130-132. In place of "glycosylation residues 2, 3 and 4...", the glycosylation residues are now designated as "...corresponding to Asn59, Asn146 and Asn 270 of SEQ ID NO:1...", thereby further defining and clarifying the claimed composition of matter.

***35 U.S.C. § 112, First Paragraph Rejections***

The Examiner has rejected claims 123-138 under 35 USC § 112 1st Paragraph, as failing to comply with the written description requirement. The Examiner's rejections are respectfully traversed. Claims 125-128 and 130-132 have now been canceled, rendering moot the Examiner's rejections thereof. Claims 123, 124, 129 and 133 have now been amended.

The Examiner has asserted that the limitation of "a set of structural coordinates of Table 4" can be interpreted to mean any two or more coordinates of Table 4, thus rendering the characteristics of the claimed composition of matter undefined and unlimited. Applicant wishes to point out that claim 123 recites "...wherein said set of structure coordinates comprises a set of structural coordinates as set forth in Table 4.", and that one of ordinary skill in the art would clearly understand that this limitation relates to the entire set of coordinates as set forth in Table 4.

In order to further clarify the characteristics of the claimed composition of matter, and in order to expedite prosecution in this case, claims 123 and 129 have now been amended to recite the phrase "...the set of structural coordinates..." rather than "...a set of structural coordinates...", thereby clearly defining the characteristics of the claimed crystalline structure and polypeptide.

The Examiner has further asserted that the recitation of "...an amino acid sequence..." renders the structure of the glucocerebrosidase polypeptide of claims

129-138 undefined and unlimited. Applicant wishes to point out that original claim 129 relates to a purified glucocerebrosidase preparation at least 95% homologous to SEQ ID NO: 1, glycosylated at Asn 19 and unglycosylated at glycosylation sites 2, 3 and 4. Thus, the Examiner's interpretation of the claimed polypeptide as being "any two or more contiguous amino acids of SEQ ID NO: 1" is inconsistent with the limitations of the claim. In order to further clarify the structure of the claimed glucocerebrosidase polypeptide or composition of matter, and in order to expedite prosecution in this case, claims 129 and 133 have now been amended to recite the phrase "...the amino acid sequence of SEQ ID NO: 1...", thereby clearly defining the metes and bounds of the claimed polypeptide.

The Examiner has alleged that the specification only discloses a single representative species of the claimed genus of crystallized glucocerebrosidase, e.g. a crystal of deglycosylated SEQ ID NO: 1 as prepared according to the method as disclosed, having the space group $C222_1$ and unit cell dimensions a=107.7 Å, b=285.2 Å, and c= 91.8 Å that diffracts X-rays to a resolution of 2.0 Å. Applicant wishes to point out that amended independent claims 123 and 129 now recite the limitations of the polypeptide being a glucocerebrosidase polypeptide, glycosylated at Asn 19, unglycosylated (defined as "fully unglycosylated" according to the specification, page 5, line 25) at at least one of Asn59, Asn146 and Asn 270. Such a polypeptide, as evidenced by the instant specification, provides a crystalline structure capable of diffracting X-rays enabling generation of of the set of structure coordinates as set forth in Table 4. It will be appreciated that the coordinates of the claimed crystallized material are inherent to the material, and would not differ from preparation to preparation. Inasmuch as claim 123 relates to the crystallized polypeptide, and claim 129 relates to the purified polypeptide having the capability of forming a crystal providing such X-ray coordinates, Applicant submits that the polypeptide, as defined in amended claim 129, and the crystalline glucocerebrosidase, as defined in amended claim 123, are fully disclosed in the instant specification.

The abovementioned notwithstanding, and in order to expedite prosecution in this case, Applicant has chosen to amend claims 123 and 129 to included the limitations of the crystallized glucocerebrosidase molecule having unit cell dimensions of a = about 107.7 angstroms, b = about 285.2 angstroms and c = about 91.8 angstroms and a crystal space group of $C222_1$, as recited in now canceled claims 125 and 126.

Further, the Examiner has rejected claims 123-138 under 35 USC § 112 1st Paragraph, as lacking enablement for making or using the invention commenurate with the scope of the claims. The Examiner's rejections are respectfully traversed. Claims 125-128 and 130-132 have now been canceled, rendering moot the Examiner's rejections thereof. Claims 123, 124, 129 and 133 have now been amended.

The Examiner has based this enablement rejection on the assertedly "reasonable" interpretation of the phrases "...a set of structural coordinates of Table 4...", and "...an amino acid sequence of SEQ ID NO: 1...", to mean any two or more x, y or z coordinates of Table 4, and any two or more contiguous amino acids of SEQ ID NO: 1. In order to further clarify and define the claimed inventions, claims 123 and 129 have now been amended to include the limitations of "...the set of structural coordinates as set forth in SEQ ID NO:1..." and "...the amino acid sequence of SEQ ID NO: 1...", thereby further defining and clarifying the claimed crystalline (claim 123) and the structure of the purified, uncrystallized (claim 129) glucocerebrosidase.

The Examiner has further asserted that one of ordinary skill in the art would not be able to make the claimed crystallized glucocerebrosidase preparation without resorting to undue experimentation. Applicant disagrees.

Although prediction *a priori* of the conditions that lead to successful crystallization of a diffraction-quality polypeptide crystal may be unlikely, the state of the art indicates that a factorial approach can be effective. While the amount of experimentation required to determine effective crystallization conditions may be significant, such work is definitely of a routine and technical nature, easily carried out by unskilled technicians or robots:

> "The most common parameters that are changed include protein concentration, the nature and concentration of the precipitant, pH, and temperature...Robotic workstations can be highly effective in conducting such multi-factorial experiments." (Drenth et al, "Principles of X-Ray Crystallography", page 8, 1.3.5).

Such robotic techniques, available at the time of filing of the instant application, can easily examine thousands of conditions for crystallization, in small volumes, per hour:

> With the advent of structural genomics has come an increase in interest in the automated setting up of large numbers of crystallization experiments. Both commercially available and

> in-house systems have been assembled to tackle this problem. Descriptions of a number of systems and approaches have been published (Oldfield et al., 1991; Soriano & Fontecilla-Camps, 1993; Sadaoui et al., 1994; Luft et al., 2000, 2001; Mueller et al., 2001; Krupka et al., 2002; Rupp et al., 2002; Santarsiero et al., 2002; Sulzenbacher et al., 2002; Brown et al., 2003; DeLucas et al., 2003; Hosfield et al., 2003; Walter et al., 2003). The dominant characteristics of these systems are that they are generally based upon use of higher density 96-, 384- or even 1536-well plates and that they use 1 ml or less of protein solution per experiment. Several systems have been described that use 100 nl or less of macromolecule solution per crystallization (Krupka et al., 2002; Stevens, 2000; Hosfield et al., 2003; DeLucas et al., 2003). Recently, a low-cost manual approach to rapidly setting up drops as small as 25 nl has been described which is suitable for laboratory use (Yeh, 2003) (Forsythe et al, Acta Cryst 2006:62:339-346).

Further, Buts et al. have concluded that although protein isoforms having variant amino acid sequences produce variant crystal strutures, crystallization success rate can be significantly improved using the information provided by their analysis:

> "A comparative analysis of the crystal-packing contacts revealed that the variable amino acids are often involved in lattice contacts and a single amino-acid substitution can suffice to radically change crystal packing. A statistical approach proved reliable to estimate the compatibilities of the variant sequences with the observed crystal forms. In conclusion, natural variation, universally present within prokaryotic species, is a valuable genetic resource that can be favourably employed to enhance the crystallization success rate with considerably less effort than other strategies. (Buts et al, Acta Cryst, 2005, Abstract)

Thus, Applicant submits that now amended claims 123 and 129 read on clearly defined glucocerebrosidase polypeptide molecules, which can form crystal structures having characteristic structural coordinates, space group and unit cell dimensions, which glucocerebrosidase polypeptides and crystals are supported, and thus fully enabled throughout the instant specification.

***102(a)/103(a) Rejections: Dvir et al. (EMBO Reports, 2003; 4:704-709)***

The Examiner has rejected claims 129-138 under 35 U.S.C. 102(a) and 103(a) as anticipated by [102(a)] or obvious [103(a)] over Dvir et al. The Examiner's rejection is respectfully traversed. Claims 130-132 have now been canceled,

rendering moot the Examiner's rejection thereof. Claims 129 and 133 have now been amended.

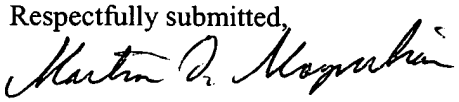
Applicants are attaching a Declaration under 37 CFR 1.132, in which Prof. Anthony H. Futerman asserts that the co-author, Andrew A McCarthy, identified along with Hay Dvir, Michal Harel, Lilly Toker, Israel Silman, Anthony H. Futerman and Joel L. Sussman in an article entitled *X-Ray Structure of Human Acid-Glucosidase, the Defective Enzyme in Gaucher Disease*, published on June 3, 2003 (online publication) in "EMBO Reports" in Vol. 4, Issue 7, at pages 704-709, was identified as a co-author on said article along with Hay Dvir, Michal Harel, Lilly Toker, Israel Silman, Anthony H. Futerman and Joel L. Sussman for his collaborative efforts operating under our guidance and direction, and was not a co-inventor of the above-identified application. The Declaration further asserts that the only inventors of the invention are Hay Dvir, Michal Harel, Lilly Toker, Israel Silman, Anthony H. Futerman, Swetlana Adamsky and Joel L. Sussman.

As such, the above-identified publication cited by the examiner to reject claims 129-138 is not a publication of "another", but rather Applicants' own publication, which was published less than a year before Applicants filing date, and thus is not proper prior art thereagainst.

Thus, Applicants respectfully request withdrawal of the rejection on the basis of 102(a)/103(a).

In view of the foregoing amendments and remarks, pending claims 123, 124, 129 and 133-138 are deemed to be allowable. Their favorable reconsideration and allowance is respectfully requested.

Respectfully submitted,



Martin D. Moynihan
Registration No. 40,338

Date: December 17, 2008

***Enclosure:***

- Executed Declaration of Anthony H. Futerman